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SHORT COMMUNICATION

Epidemiology of soil transmitted helminth and *Strongyloides stercoralis* infections in remote rural villages of Ranomafana National Park, Madagascar

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ABSTRACT

Soil-transmitted helminth (STH) infections carry the highest number of disability adjusted life years among all neglected tropical diseases, disproportionately affecting low-income countries such as Madagascar. This study describes the epidemiology of STH and *S. stercoralis* infections in twelve remote villages surrounding Ranomafana National Park (RNP), Ifanadiana, Madagascar. Questionnaires and stool samples were collected from 574 subjects from random households. The Kato-Katz method and spontaneous sedimentation technique were used to examine stool samples for evidence of infection. Infection prevalence rates were 71.4% for *Ascaris lumbricoides* (95% CI: 67.7–75.1), 74.7% for *Trichuris trichiura* (95% CI: 71.1–78.2), 33.1% for hookworm (95% CI: 29.2–36.9), and 3.3% for *Strongyloides stercoralis* (95% CI: 1.84–4.77). Participants who were older in age (OR = 0.96; 95% CI: 0.95–0.99) and who had a high school education (OR = 0.17; 95% CI: 0.04–0.77) were less likely to be infected with a STH. Females were less likely to be infected with *A. lumbricoides* (OR = 0.52; 95% CI: 0.33–0.82). Participants living in villages further from the main road were more likely to be infected with a STH (F = 4.00, p = 0.02). Overall, this study found that 92.5% (95% CI: 90.3–94.6) of the people living in rural regions near RNP have at least one STH infection. This calls into question the current preventative chemotherapy (PC) program in place and suggests that further medical, socioeconomic, and infrastructural deveopments are needed to reduce STH prevalence rates among this underserved population.

KEYWORDS
Soil-transmitted helminth; *Strongyloides stercoralis*; Madagascar; Ranomafana; intestinal parasites; Ifanadiana

Introduction

Soil transmitted helminths, including *Ascaris lumbricoides, Trichuris trichiura*, and hookworm species *Ancylostoma duodenale* and *Necator americanus*, affect more than 1.0 billion people globally. Approximately 5.3 billion people worldwideare at risk of STH transmission, of which 1.0 billion are school-aged children [1]. Combined, STH infections cause the largest disability adjusted life years (DALYs) loss among all Neglected Tropical Diseases [2]. *Strongyloides stercoralis* is often underreported due to the limited ability of the most commonly used STH diagnostic technique, the Kato-Katz method, to detect this particular parasite [3].

STH and *S. stercoralis* infections have an especially devastating impact on children due to associated malnutrition, growth stunting, and cognitive and developmental delay [4–6]. Additionally, anemia caused by hookworm infections negatively affects the productivity and wage-earning potential of adults [5].

Madagascar is the fifth poorest country in the world and has 9 million children (pre-school and school-aged) who are at risk for intestinal parasite infections [7]. The population near Ranomafana National Park (RNP) in the Ifanadiana district of Madagascar has significant poverty and malnutrition, with 61% of children being underweight and 72% having signs of stunted growth [8]. The most recent STH infection prevalence study from the Ifanadiana district was conducted in 1990–1992and showed infection rates of 78% for *A. lumbricoides*, 38% for *T. trichiura*, and 16% for hookworm in children under 11 years old. The prevalence of *S. stercoralis* was not reported [9]. In the elapsed time since this study, there has been a global effort to treat and prevent these infections through the deployment of mass drug administration/preventive chemotherapy (MDA/PC) campaigns. The WHO recommends biannual MDA/PC for STH infections in preschool, school-aged children, pregnant women, and adults who are

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constantly exposed to STH. However, WHO recommendations do not reach all areas of Madagascar [10].

The objective of this study is to provide a much-needed report on the prevalence rates of STH and *S. stercoralis* infections and to identify the risk factors among a neglected population living in remote villages neighboring RNP in Madagascar.

Material and methods

Study area and population

Twelve villages (Ambinanindranofotaka, Ampitambe, Ampitavanana, Ankazotsara, Bevoahazo, Kianjanomby, Mandrivany, Mangevo, Marozano, Sahavanana, Sahavoemba and Torotosy) on the periphery of Centre ValBio (CVB), anonprofit research and environmental conservation center affiliated with Stony Brook University located near RNP, Madagascar, 47°E, 21°S, (Figure 1), were selected for this study. The distance to the main road from each village was measured using Google Earth geographic information system (Mountain View, CA, USA). The participating villages were found to be traditional, rural, and heavily reliant on subsistence agriculture. Village sanitation is poor, with lack of water infrastructure and commonly practiced indiscriminate defecation. All twelve villages cannot be reached by motor vehicle travel since there are no roads or paved paths.

Study design

A cross-sectional study was conducted in the twelve highly remote villages within the Ifanadiana district of Madagascar from the months of June to August 2016. Households were randomly selected, using an electronic random number generator on the village census. All household members five years of age and older (if younger than 18, with parental permission) were invited to participate. Participating individuals were interviewed in the Malagasy language in order to complete a questionnaire. The questionnaire inquired about demographics, handwashing frequency, traditional healer visitation, anti-parasitic medication source and frequency, history of worm defecation, and awareness of parasitic infections.

Stool collection and parasitological examination

Each participant was instructed to collect a thumb sized sample of their own stool and place it in a supplied 50 mL cup, sealing it with an airtight cap. All samples were fixed in 10% formalin solution within 24 hours of sampling. The stool samples were analyzed within 15 days of collection using two techniques, the Kato-Katz method (KKM) and the spontaneous sedimentation technique (SST) [11–13]. The intensity of infection, typically estimated when using the Kato-Katz method, could not be measured due to dilution by the addition of formalin. The preservation of stool with formalin was necessary due to the lack of basic resources such as running water and electricity in the remote villages, and the long distance to the CVB laboratory.

Statistical analysis

Analysis of Variance (ANOVA) was used to compare mean STH infection rates among participants grouped

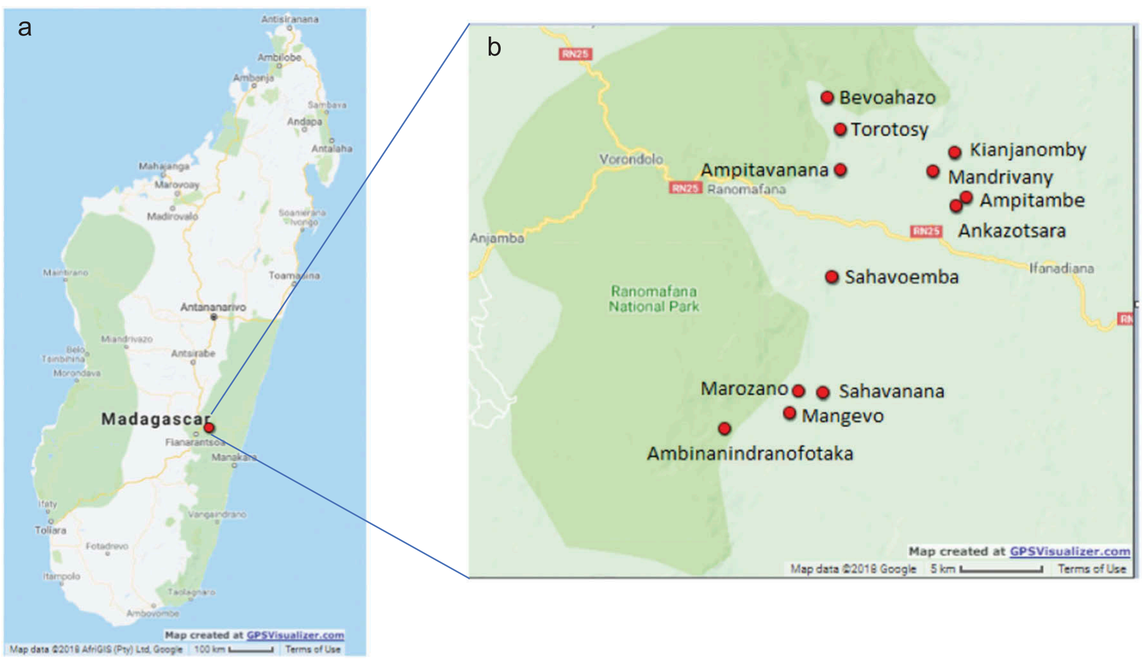


Figure 1. Map of research area in Ifanadiana District, Madagascar. Twelve Villages surveyed in the following Zones: Madrivany, Bevoahazo (excluding Amodivoangy and Fohabe), and Mangevo.

by village distance from the main road. Bivariate analysis was performed using the chi-squared test for categorical variables. *t*-test and Wilcoxon Rank Sum tests were used for continuous variables. Multivariable logistic regressions predicting infection by the parasites *A. lumbricoides, T. trichiura*, and hookworm infections while controlling for possible confounding factors were constructed. Multivariable logistic regressions were not created for *S. stercoralis* due to the low prevalence of infection. Covariates of interest were included in multivariable logistic regressions based on the bivariate analysis. The threshold for inclusion was $p < 0.10$. Cluster-robust standard errors, clustered on household, were included in these regressions. A p value < 0.05 was considered statistically significant. For parasitological technique comparison, a gold standard test was defined using the total number of positive samples for a particular parasite by any parasitological technique. Statistical analysis was conducted using STATA version 14 software.

Ethical considerations

The study was approved by the Institutional Review Board (IRB) at Stony Brook University, the National Ethics Committee of the Ministry of Public Health of Madagascar (N°089 MSANP/CE), and the IRB at Center ValBio in RNP. The study was explained to all adult participants, children, and/or children's guardians before participation. Participants and/or guardians then signed a letter of consent before inclusion in the study. Children who participated signed a letter of assent.

Results

Demographics

A total of 574 participants (52.3% males, 47.7% females) from 164 random households were included in the study. The study sample consisted of 11% of the total population from each village. Out of the 574 participants, 235 (41.0%) were children between the ages of 5–14. The median age was 19 and the range was between 5–82 years old (Table 1). The majority of the population surveyed were subsistence farmers who had reported an annual median house-hold income of 34 USD with a range of 0–1025 USD. The communities did not have access to clean water, rather utilized fresh water rivers for their basic needs, and had no electricity. They relied on open fires for heat, decontaminating water through boiling, and cooking.

Prevalence of soil transmitted helminth infections

The overall infection prevalence rates were 71.4% for *A. lumbricoides* (95% CI: 67.7–75.1), 74.7% for

Table 1. Demographics of the population around Ranomafana National Park and Prevalence of STH and *S. stercoralis* infections.

Category	Participants surveyed N (%)	Ascariasis (%)	Trichuriasis (%)	Infecting Species Hookworm (%)	Strongyloidiasis (%)	STH or Strongyloidiasis % (± 95% CI)	> 1 infecting species % (± 95% CI)
Gender							
Male	299 (52.3)	74.9	73.9	36.4	3.3	92.3 (89.6–95.2)	71.5 (66.0–76.3)
Female	272 (47.6)	67.6	75.7	29.7	3.3	92.6 (89.5–95.7)	62.5 (56.7–68.2)
Age groups in years							
5–14	235 (41.0)	72.7	84.6	36.1	2.1	97.0 (94.9–99.0)	74.0 (68.4–79.6)
15–24	108 (18.8)	72.2	75.9	37.9	5.5	93.5 (88.8–98.1)	65.7 (56.7–74.7)
25–34	85 (14.8)	67.0	74.1	31.7	1.1	92.9 (87.4–98.4)	63.5 (53.2–73.8)
35–44	62 (10.8)	67.7	53.2	22.5	1.6	83.8 (74.6–93.1)	50.0 (37.4–62.5)
>44	82 (14.2)	73.1	60.9	26.8	7.31	84.1 (76.1–92.1)	63.4 (53.0–73.9)
Education							
None	91 (15.9)	75.8	73.6	25.3	4.40	90.1 (83.9–96.2)	63.7 (53.8–73.7)
Elem. School	388 (67.6)	71.9	73.7	35.3	3.09	93.6 (91.1–96.0)	67.5 (62.9–72.2)
High School	27 (4.70)	48.1	66.7	18.5	0.0	81.5 (66.5–96.4)	40.7 (21.9–59.6)
College	1 (0.17)	0.0	100.0	0.0	0.0	100.0	0
Unknown	67 (11.7)	73.1	85.1	37.3	4.48	94.0 (89.0–99.0)	79.1 (69.3–88.9)
Village Distance from main road							
0–5 km	270 (47.0)	62.6	68.9	36.7	2.60	89.6 (86.1–93.1)	59.6 (53.8–65.4)
5–10 km	160 (27.9)	73.8	78.8	33.8	1.25	93.1 (92.2–96.0)	70.6 (63.5–77.7)
10–15 km	144 (25.1)	85.4	61.8	25.7	6.94	97.2 (94.5–99.9)	76.4 (69.4–83.4)
Total: N (%, 95% CI)	**574 (100)**	**410 (71.4, 67.7–75.1)**	**429 (74.7, 71.1–78.2)**	**190 (33.1, 29.2–36.9)**	**19 (3.31, 1.84–4.77)**	**531 (92.5, 90.3–94.6)**	**66.9 (63.0–70.8)**

Percent is based out of total number of participants. Some participants did not respond to all survey questions asked. STH = Soil Transmitted Helminth. CI = Confidence Interval

T. trichiura (95% CI: 71.1–78.2), 33.1% for hookworm (95% CI: 29.2–36.9), and 3.31% for *S. stercoralis* (95% CI: 1.84–4.77) (Table 1). In children between 5–14 years old, the overall prevalence of *A. lumbricoides* was 72.4%, *T. trichiura* was 84.6%, hookworm was 36.1%, and *S. stercoralis* was 2.1%.

Risk factors

Our results indicate that age, level of education, sex, and village distance from a main road were associated with the prevalence of STH infections. For every one-year increase in age (x vs. x + 1, where x = age), there was a decreased risk in having any STH infection (OR = 0.96; 95% CI: 0.95–0.99; p = 0.02) and a decreased risk of being infected by T. *trichuris* (OR = 0.98; 95% CI = 0.97–0.99; p = 0.01) after controlling for covariates. Participants with a high school education were less likely to have any STH infection (OR = 0.17; 95% CI: 0.04–0.77; p = 0.02) and less likely to have an *A. lumbricoides* infection (OR = 0.28; 95% CI: 0.08–0.94; p = 0.04) compared to participants with no formal education after controlling for covariates. Female participants had a decreased risk of being infected with *A. lumbricoides* compared to males (OR = 0.52; 95% CI = 0.33–0.82; p < 0.01) after controlling for covariates (Table 2).

Participants living within 5 km of the main road were found to have lower rates of infection (89.6% CI: 86.1–93.1), than those both living within 5–10 km (93.1% CI:92.2–96.0) and greater than 10 km (97.2% CI: (94.4–99.9)) (F = 4.0, p = 0.02) from the main road (Table 1). Likewise, individuals infected by any STH were found to be living significantly further away from the main road (mean: 6.3 km) than those not infected (mean: 4.5 km) (p = 0.002). Not surprisingly, there was a positive correlation between having a STH infection and living further from the main road ($R^2 = 0.57$) (Figure 2).

Consistent with the above results, multiple specie infections (>1 STH infection) were found more prevalent in males (p = 0.02), people under 35 years of age (p < 0.01), and non-high school educated individuals (p = 0.03). Participants that live greater than 5 km from the main road were also found to have a higher prevalence of multiple specie infections when compared to individuals living less than 5 km from the main road (p < 0.01) (Table 1).

Questionnaire responses

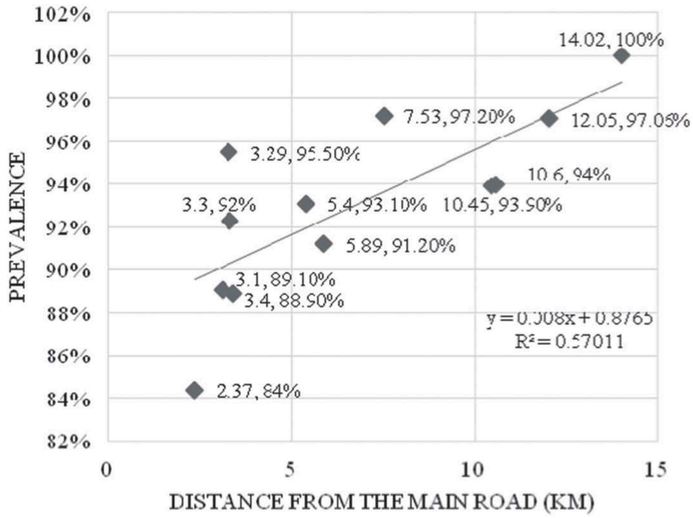
Only 12.7% (95% CI: 9.6–15.8) of participants reported being aware of how STH infections could be spread, despite 83.2% (95% 80.2–86.2) that admitted to defecating at least one worm in their lifetime. With regards to hygiene, nearly 93% reported washing their hands at least one time each day, with the mean and median number of hand washings being 3.2 (SD = 1.6) and 3 (Range 0–10), respectively. However, due to a reported lack of access, 60.0% (95% CI: 44.9–74.9) of participants do not always use soap, and 32.0% (95% CI: 17.9–46.1) reported never using soap to wash their hands.

Each participant was questioned about the type of anthelminthic medication they consume, the frequency of consumption, and where the medication was acquired. 83.5% of participants reported using only albendazole to treat helminthiasis, 0.5% use praziquantel alone, 3.3% use both albendazole and praziquantel, 2.1% consume some ‘other’ medication for parasitic relief and 10.6% chose not to respond. With

Table 2. Multivariable logistic regression predicting STH infection.

Category	Odds Ratio [95% CI] (p-value)			
	Ascariasis	Trichuriasis	Hookworm infection	Any STH infection
Gender				
Male	*	-	*	*
Female	0.52 [0.33–0.82] **(<0.01)	-	0.79 [0.54–1.16] (0.23)	0.52 [0.23–1.18] (0.11)
Age				
X (where X is age)	-	*	-	*
X + 1 (where X is age)	-	0.98 [0.97–0.99] **(0.01)	-	0.96 [0.95–0.99] **(0.02)
Education				
None	*	-	*	*
Elementary School	0.73 [0.41–1.3] (0.3)	-	1.55 [0.94–2.56] (0.09)	1.55 [0.48–5.04] (0.46)
High School	0.28 [0.08–0.94] **(0.04)	-	0.62 [.56–2.46] (0.5)	0.17 [0.04–0.77] **(0.02)
Medication Source				
School	-	-	-	*
Free Clinic	-	-	-	0.14 [0.02–1.25] (0.08)
Self-Purchase	-	-	-	0.17 [0.64–1.32] (0.67)

*Household cluster-robust standard error. CI: Confidence Interval STH: Soil Transmitted Helminth infection; X = age;X + 1 = Reference age +1 year; * = reference; ** = significant value, p < .05*



Village Key	
A	Ankazotsara (2.37km)
B	Ampitavanana (3.1km)
C	Ampitambe (3.29km)
D	Sahavoemba (3.3km)
E	Mandrivany (3.4km)
F	Torotosy (5.4km)
G	Kianjanomby (5.89km)
H	Bevoahazo (7.53km)
I	Marozano (10.45km)
K	Mangevo (12.05km)
L	Ambinanindranofotaka (14.02km)

Figure 2. Village distance from the main road (km) vs. STH prevalence (%).

regards to the frequency of medication use, 78% of the respondents stated that they consume antiparasitic medications twice a year, 11.7% consume medications as needed, 1% consume them only when prescribed, 1.5% never consumed antiparasitic medications, and 7.8% of participants chose not to respond. About 47.1% of respondents stated that their anthelminthic medications are delivered to their house, 19.4% obtain medications from the local school, 17.2% buy medications from a store, 6.2% obtain medications from a health clinic, 2.6% acquire medications from multiple sources, and 7.6% of participants chose not to respond.

Local health officials were also asked about MDA/PC protocols in the area and they reported that they follow local guidelines that state all persons greater than 2 years old should receive 400 mg of albendazole or 500 mg of mebendazole once a year for helminthiasis prevention and treatment.

Discussion

The prevalence of STH and *S. stercoralis* infections in the remote villages surrounding RNP is overwhelmingly high with 92.5% (95% CI: 90.3–94.6) of the sampled population having at least one type of STH infection. *A. lumbricoides* and *T. trichiura* are infecting more than two thirds of the population indicating that fecal-oral contamination is high in these communities. These results show a lack of progress in reducing the STH infection burden over the past two decades, as prevalence rates are nearly as high or even higher than the last STH infection prevalence study in the region in 1992 [9].

Our results show that 78% of the entire population studied and 84% of school-aged children, consume anthelminthic medication twice a year on average. Surprisingly, local health officials only report MDA/PC once per year in this region. This suggests that the villagers are receiving medications outside of the PC protocols. The majority of participants stated that they acquired their medication from a source other than the government, their schools, or health clinics. Anthelmintic medications are readily available in the Ranomafana region, but the quality and identity of these medications is not known. In this region, traditional herbal remedies are also used to treat STH infections [8], and it is likely that our research participants use alternative therapies to treat their condition. It is important to note that while a single dose of mebendazole may be sufficient for treating ascariasis and hookworm infections, effective trichuriasis treatment requires three doses of anthelmintic medication. In addition, in areas where STH prevalence rates are greater than 50%, all individuals who work as farmers should be receiving a minimum of two annual doses of mebendazole [10,14]. Moreover, the first line treatment for *S. stercoralis* infections is a single dose of Ivermectin for 1–2 days [15]. All of this suggests that the current MDA/PC protocol is likely insufficient in preventing and eliminating all STH infections and does not address *S. stercoralis* infections.

In addition to inadequate treatment, our results reveal a lack of higher education and resources among the studied population. Out of the participants, 12.7% (95% CI: 9.6–15.8) reported not knowing how STH infections are spread. Despite this, the vast majority of participants appear to wash their hands at least three times per day on average, albeit usually without soap and using local rivers, streams, or rice paddies. A reported lack of resources, including soap, not to mention clean water and basic hygiene facilities, leading to the widespread practice of open defecation, are likely major contributors to the high STH prevalence rates found in this area. While it is appropriate to wonder if the quality or quantity of the anthelmintic medication this population attains could be improved, it is evident that a lack of resources and education are driving these prevalence rates to extraordinary heights. Further studies are required to more clearly answer these questions, but perhaps the most pressing matter is the need for socioeconomic and infrastructural change to reduce disease risk and burden.

Age, gender, education, and distance to a main road were individually found to be associated with the probability of having an STH infection. After controlling for factors such as education and source of anthelminthic medication, our study found that younger age is associated with a higher STH infection rate, especially *T. trichiura* infections. This may be related to several factors previously hypothesized during studies of other rural regions, including the increased exposure of children to STH contaminated soils. For example, studies in both China and Malaysia found that younger individuals were more likely to have parasitic infections [16,17]. In our study, males were found to have higher rates of *A. lumbricoides* infections. One study conducted in Cameroon found increased rates of *T. trichiura* in men, although they did not note a gender-based difference with other STH infections such as *A. lumbricoides* [18]. A possible explanation for our results is the differing gender roles among our study population. While men typically work in rice paddies with more opportunities for indiscriminate defecation and less access to soap, women typically manage child-rearing at home with easier access to latrines, therefore may have less overall exposure to STHs. Increased education was also found to protect against infection likely due to increased awareness of methods of transmission or increased disposable income to acquire medications and healthcare. Public health authorities have been targeting school aged children for anthelminthic treatment due to the overall higher prevalence of STH infections and greater morbidity associated with acute or chronic infection [19]. However, if STH infections remain prevalent amongst family members and fellow villagers in a setting where sanitation is poor, it is no surprise that children are maintaining high rates of infection.

Our study found that distance from a main road was also associated with STH infections. The population of STH infected individuals were on average significantly further from the main road than the population of non-infected individuals. In addition, a direct relationship can be visualized between the distance from the main road and the overall STH infection prevalence in the village population (Figure 2). It is likely that STH knowledge, access to therapeutic medications, and healthcare interventions including MDA/PC programs are more deficient when the community is more remote. The challenges healthcare workers face when attempting to reach remote communities may adversely affect their ability to implement MDA/PC programs.

In this study, participant stool samples were preserved in formalin between collection and microscopic examination. Although formalin is adequate in preserving parasite eggs [20], hookworm eggs are particularly vulnerable to degradation [21]. The reported hookworm prevalence of 33.1% may underestimate the actual prevalence in the population studied. In addition, the prevalence of other STH infections may be underestimated as a result of the use of formalin in preservation and the delay in sample analysis. Unfortunately, our use of formalin prevented the quantification of parasite burden permitted by the Kato Katz technique. However, formalin preservation was necessary to collect and transport stool samples from these remote communities. The reported *S. stercoralis* prevalence is also likely underestimated, as techniques such as the Baermann technique are more sensitive and were not utilized. This study is unique because it examines the prevalence and risk factors in truly remote, roadless Madagascan villages. STH infection prevalence is frequently studied around the world, but rarely in such difficult to access areas.

In conclusion, despite MDA/PC efforts, the prevalence rates of STH infections including *S. stercoralis* in rural areas of Madagascar remain extraordinarily high. These results suggest that the current MDA/PC program may not be optimized to prevent and treat all STH infections in this region. Further evaluation is necessary to help guide future protocols and to help reduce the burden of STH infections. Additionally, there is an eminent need for socioeconomic and infrastructural improvements in the region, or we fear that disease burden and its consequences will remain high among this population.

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Disclosure statement

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